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Rapid Lactate Determination with an Electrochemical Enzymatic Sensor: Clinical Usability and Comparative Measurements

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Summary: Lactate measurements can be performed within 2–3 minutes after blood withdrawal from the patients by using an electrochemical enzymatic sensor for lactate. The values obtained reflect the actual state of the patient which is not the case with the slow classical method using lactate dehydrogenase and NAD.

The sensor is reproducible and the influence of the main reducing substances found in the blood is small enough to be of no clinical significance. Drugs commonly used in intensive care stations have no influence on the sensor. In vitro lactate production of the blood cells has been studied under various conditions.

66 pairs of comparative measurements between the classical method and the lactate sensor resulted in a good correlation coefficient.

Schnelle Bestimmung von Lactat mit einem elektrochemisch-enzymatischen Sensor: Klinische Brauchbarkeit und vergleichende Messungen

Zusammenfassung: Die Lactatbestimmung im Blut innerhalb von 2–3 Minuten nach Blutabnahme wird durch einen elektrochemisch-enzymatischen Sensor ermöglicht. Die erhaltenen Werte geben den tatsächlichen Zustand des Patienten wieder, was mit der langsamen, klassischen Methode unter Verwendung von Lactatdehydrogenase und NAD nicht möglich ist.

Die mit dem Lactatsensor erhaltenen Werte sind reproduzierbar. Der Einfluß der wichtigsten reduzierenden Substanzen im Blut ist sehr gering und nicht von klinischer Bedeutung. Medikamente, die in der Intensivpflege oft verwendet werden, haben keinen Einfluß auf den Sensor. Die in vitro Lactatproduktion der Blutkörperchen wurde unter verschiedenen Bedingungen untersucht.

Es wurden 66 Vergleichsmessungen zwischen der klassischen Methode und dem Lactatsensor durchgeführt. Der Korrelationskoeffizient ist gut.

Introduction

Many critically sick patients develop acidosis as a result of profound respiratory, hemodynamic and/or metabolic abnormalities. Plasma lactate elevations commonly result from metabolic disturbances producing acidosis particularly with associated vascular collapse. Measurement of serum or blood lactate would be of great help in distinguishing lactic acidosis from other causes and in the following treatment. Even though lactic acidosis is found in a variety of clinical circumstances, serum lactate levels are obtained much less frequently than are the other measurements of the acidotic state such as pH and carbon dioxide tension.

A combination of problems generally makes the procedure for lactate determination too expensive to perform regularly for sick patients. Lactate is being formed in vitro so any delay between drawing the sample, separating the red cells and stopping their metabolism will increase the lactate levels. In most laboratories lactate is not determined routinely so that even "stat" samples may be delayed long enough that the results no longer accurately reflect the patient's state.

Some delay is also unavoidable because of the time required to perform a single lactate determination. Since lactate levels are of greatest use for critically ill patients, the determination must reflect rapidly changing clinical

conditions and must be compatible with the work schedules of a busy staff. A technically simple device which would rapidly measure lactate would solve these problems, particularly if it were simple enough to be actually used by the staff caring for the patient. It is our purpose to describe our experience with a semi-automatic device which can be used to determine lactate levels in a clinical setting by personnel not specifically trained for the task.

Sensor

D. L. Williams et al. (4) were the first to report lactate measurements using an electrochemical enzymatic sensor. This method was later improved by *Ph. Racine* (5, 6, 7) and coworkers. With the device used here, lactate measurement is based on the specific and irreversible oxidation of *L*-lactate to pyruvate in the presence of an electron acceptor (hexacyanoferrate(III)) and the enzyme cytochrome b_2 from baker's yeast (*Saccharomyces cerevisiae*):

$$(\text{Lactate})^{-1} + 2\text{Fe}(\text{CN})_6^{-3} \xrightarrow{\text{cytochrome } b_2} (\text{Pyruvate})^{-1} + 2\text{H}^+ + 2\text{Fe}(\text{CN})_6^{-4}$$

The hexacyanoferrate(II) is reoxidized at a platinum electrode biased at 0.25–0.40 volts against a silver-silver chloride electrode.

$$2\text{Fe}(\text{CN})_6^{-4} \xrightarrow{\text{Pt}} 2\text{Fe}(\text{CN})_6^{-3} + 2e^-$$

Thus in response to the presence of *L*-lactate, the sensor develops a current which is linearly related to the external concentration of this substrate.

A scheme of the sensor is shown in figure 1.

It consists of a slightly recessed disc-shaped platinum electrode covered with a layer of enzyme solution. A semipermeable cellophane membrane separates the enzyme layer from the test solution allowing diffusion of lactate and hexacyanoferrate(III) but preventing enzyme from leaking into the test solution. As counter electrode, a silver-silver chloride electrode separated from the test solution by a glass-frit is used.

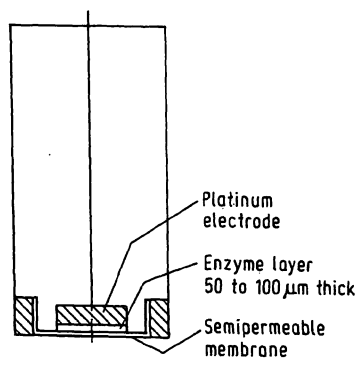

Fig. 1. Scheme of the lactate sensor

Using membranes made from regenerated cellulose (DUPONT PUDO 193) and enzyme solutions of about 2000 kU/l (at 25 °C) the sensor gives a linear calibration curve for about 4 weeks in the range 0–1.5 mmol/l. The response time of a sensor varies initially from 40 secounds at the end of its useful life time.

The intrinsic reproducibility of the sensor response is good, as can be seen from the recordings of its output current for two different lactate standards (fig. 2). A deviation not exceeding 1% is routinely observed during daily calibrations of the instrument.

When the sensor response ceases to be linear or becomes too slow due to the inevitable denaturation of the enzyme, the membrane is discarded and the platinum surface cleaned by an anoding etching. Fresh enzyme suspension (1 µl) kept in saturated ammonium sulfate is then placed on the platinum electrode and a new membrane is fitted after it has been soaked in bi-distilled water for at least one minute. The sensor can thus be used for several weeks to perform several hundred lactate assays.

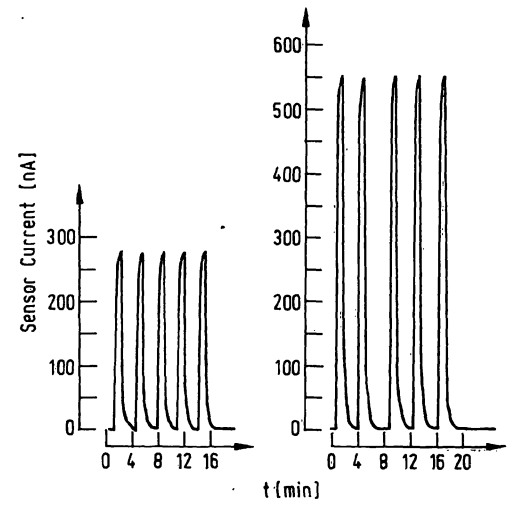

Fig. 2. Recording of the sensor current after the injection of 0.5 (left) and 1.0 (right) mmol/l lactate standard.

Instrument and Sample Measurement

A prototype of a semi-automatic Lactate Analyzer has been developed and its functions have been described in a recent publication (8). In the study reported here, an earlier laboratory prototype was utilized whose essential functions do not differ from the latest unit. The heart of the instrument is a plexiglass measurement cell containing the sensor and the counter electrode. The total volume from sample inlet to outlet is 100 µl and the volume between the electrodes (70 µl) is thermostated at 20 °C. Figure 3 is a schematic diagram of the measurement system and figure 4 a picture of the instrument. Emptying and washing of the cell is automatic and the readout is analog. Calibration is performed

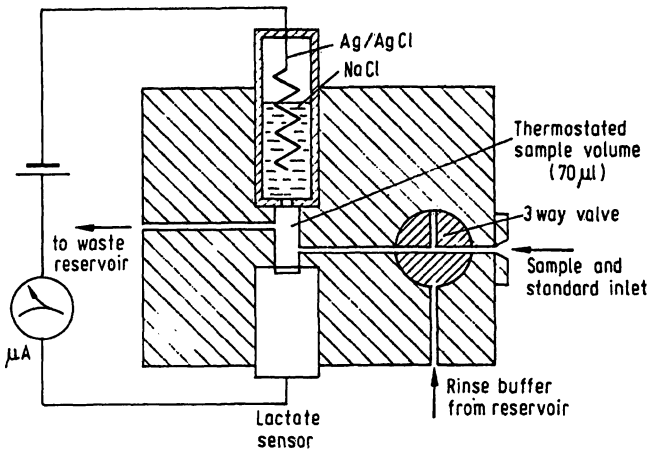

Fig. 3. Schematic diagram of the measurement system

twice daily with a standard of 1.00 mmol/l by adjusting the readout on 10.0 mmol/l with the calibration potentiometer. Linearity is then checked with a standard of 0.50 mmol/l.

Physiological lactate levels can be above 10 mmol/l, thus the sample is diluted 10 times prior to measurement. A sample of at least 50 µl of whole blood is taken and diluted 10 times with an isotonic phosphate buffer (pH = 7.3, 0.10 mol/l + 2 g/l NaF + 1 g/l NaN_3). 0.50 ml of this diluted sample are injected into the measurement cell. The same volume of standard is used for calibration. Between measurements the cell is rinsed using a solution of 0.2 mol/l phosphate buffer (pH = 7.3) containing 2.0 mmol/l hexacyanoferrate(III).

Method

Sixty-six comparative measurements of blood were used to calculate the correlation between the enzymatic method performed in the laboratory of the "Medizinische Universitätsklinik, Tübingen" and the Lactate Analyzer. The enzymatic spectrophotometric determinations were performed according to the method of *Hohorst* (12), which was checked by *Linden* (13).

The lactate determination with the Lactate Analyzer was performed according to the above method. Immediately after withdrawal, 100 µl whole blood were diluted 10 times with the isotonic phosphate buffer (0.1 mol/l pH = 7.3). 0.50 ml of this diluted sample were injected into the measurement cell using a disposable 2 ml-syringe. The readout followed between 40–60 seconds. Thereafter and just before the next measurement was started the cell was rinsed with 0.2 mol/l phosphate buffer, pH = 7.3, containing 2.0 mmol/l hexacyanoferrate(III). Each sample was determined twice and the mean value is shown in table 1.

Blood was withdrawn, without heparin addition, from a freely flowing brachial vein, a femoral artery or from the pulmonary artery during cardiac catheterization. The samples were processed immediately. In 7 cases it was not possible to perform the lactate determination immediately. Five of these were diluted in phosphate buffer + sodium fluoride (2 g/l) at a ratio of 1:10 and stored in an ice bath. For two, the blood was heparinized and placed in the ice bath immediately. These 7 samples were chemically analyzed between 20 and 60 minutes following blood withdrawal.

Blood cells continue to produce lactate when citrated or heparinized blood is allowed to stand at room temperature. Furthermore during a series of preliminary measurements it had been observed that lactate also rose when diluted blood samples were left standing at room temperature. To test this observation and to get an optimum determination of lactate, 10 ml heparinized blood was taken from 7 subjects. From each sample, 3 ml of the blood were poured immediately into a second tube. Two ml of blood were pipetted into a third and fourth tube which contained 8 ml of 0.1 mol/l phosphate buffer + 2 g/l NaF. The second and fourth tubes were immediately placed into an ice

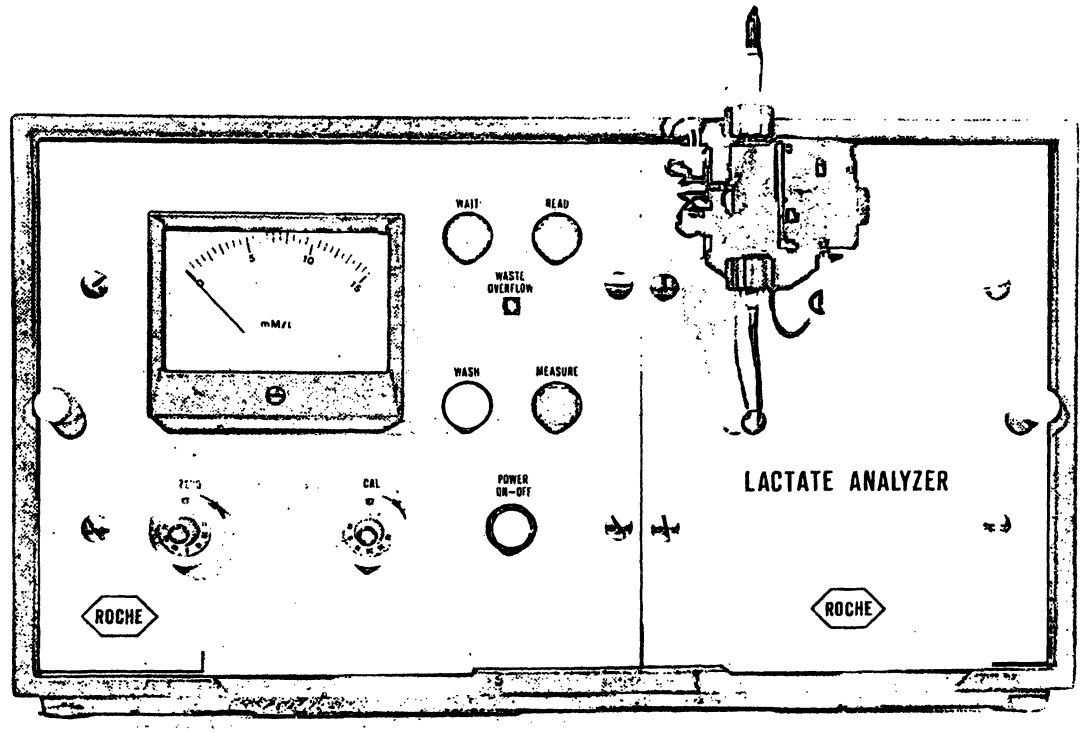

Fig. 4. Prototype of the Lactate Analyzer

Tab. 1. Lactate values obtained with the classical method and the Lactate Analyzer

No.	Spectrophotometric method [mmol/l]	Lactate Analyzer [mmol/l]	No.	Spectrophotometric method [mmol/l]	Lactate Analyzer [mmol/l]	No.	Spectrophotometric method [mmol/l]	Lactate Analyzer [mmol/l]
1	1.900	1.900	24	1.780	1.780	48	0.790	0.700
2	0.900	1.000	25	0.640	0.790	49	1.150	0.950
3	0.980	1.200	26	1.920	1.900	50	1.000	0.820
4	1.540	1.800	27	1.980	2.000	51	1.210	1.050
5	1.180	0.950	28	0.950	0.950	52	0.930	0.800
6	1.440	1.100	29	0.610	0.850	53	0.920	0.780
7	1.220	0.950	30	3.640	3.900	54	0.830	0.750
8	0.880	0.600	31	2.510	2.100	55	0.810	0.700
9	0.570	0.450	32	4.470	4.250	56	0.790	0.700
10	0.980	0.650	33	2.060	2.000	57	0.800	0.680
11	0.860	0.600	34*	7.640	7.150	58	0.790	0.690
12	0.830	0.600	35	3.890	4.050	59	0.880	0.820
13	1.300	1.300	36	1.620	1.700	60	1.010	0.900
14	1.200	0.900	37	1.020	0.910	61	1.030	0.900
15	0.930	0.800	38	0.870	0.880	62	1.170	1.050
16	1.230	1.000	39	0.730	0.800	63	0.740	0.700
17	0.670	0.500	40	0.780	0.780	64	0.830	0.780
18	0.650	0.500	41	0.720	0.720	65	0.770	0.650
19	0.620	0.950	42	0.860	0.700	66	0.760	0.780
20	1.150	1.200	43	0.870	0.700			
21	0.630	0.650	44	0.850	0.800			
22	2.440	2.560	45	1.010	0.850			
23	4.170	4.400	46	0.980	0.920			
			47	1.120	1.000			

* Error in the setting of the dilution ratio

bath. The first and third tubes were kept at room temperature. The first measurement of lactate was started within 4 minutes after blood sampling. The lactate concentrations from tubes 1, 2, 3 and 4 were sequentially measured at 3–4 minute intervals until the 72nd minute. Thereafter, measurements were made at the 80th, 90th, 110th and 120th minute. The dilution ratio for these measurements was 1:5 (blood: 0.1 mol/l phosphate buffer). Calibrations were made before starting the measurements, in the 75th minute and after their completion. All measurements were made with the Lactate Analyzer according to the above method.

The reaction catalyzed by cytochrom b_2 is specific for *L*-lactate, but other substrates can react with reduced efficiency (9, 10). The most active are α-hydroxy-*n*-butyrate, α-hydroxy-*n*-caproate and α-hydroxy-isocaproate. Only β-hydroxy-*n*-butyrate is encountered in blood where it reaches concentrations of 10 mmol/l in diabetic patients. To test this, 0.1, 1.0 and 10 mmol/l β-hydroxy-*n*-butyrate (Merck) were prepared in a 0.1 mol/l phosphate buffer at pH of 7.3. These were compared with a 1.0 mmol/l lactate standard prepared in the same buffer.

It has been reported (6) that blood reducing substances are capable of either reducing the acceptor, hexacyanoferrate(III) or being oxidized at the platinum electrode. Some of these substances were added to the 10 mmol/l lactate standard and the resulting changes determined. These are shown in table 4. Low concentrations of certain drugs can act as inhibitors or activators of enzyme activity. Drugs often prescribed for critically ill patients were tested using a 1.0 mmol/l lactate standard to which the drug was added. Table 5 shows the concentrations of these drugs.

Results

All comparative measurements are given in table 1. Table 2 shows the correlation coefficient and the regression equation for these values. There is a good correlation (r = 0.990). Figure 5 shows the regression line with the 95% confidence level for the data points.

Tab. 2. Correlation coefficient for the comparative measurements

Number of measurements pair	66
Correlation coefficient	0.990
Regression line	
– Intercept a_0	– 0.055 ± 0.032
– Slope a_1	0.988 ± 0.018

The results of incubation measurements are given in table 3 and plotted on figure 6. The mean percent increase ± the standard deviation from the first measurement is shown for the 7 samples. The initial lactate values were between 0.6 and 2.0 mmol/l. The results show that there is a continuous and nearly linear increase in lactate concentration when heparinized blood is stored at room temperature. However, if heparinized whole blood is placed in an ice bath, there is no increase in lactate concentration over 120 minutes.

Blood diluted with buffer containing NaF and stored at room temperature showed an initial increase in measured lactate. This increase was more rapid than the increase observed in undiluted blood during that period of time ($p < 0.05$). The rapid increase in apparent lactate level stops after 20–30 minutes. When heparinized blood

Tab. 3. In vitro effect of temperature and dilution in NaF containing buffer on blood lactate levels

Heparinized whole blood at room temperature (23 °C)			Heparinized whole blood in ice bath		
Incubation time [min]	Lactate increase [%] $\bar{x} \pm s$	Significance level	Incubation time [min]	Lactate increase [%] $\bar{x} \pm s$	Significance level
17	13.8 ± 3.74	$2p < 0.001$	7	- 4.8 ± 8.53	$2p < 0.2$
32	20.6 ± 4.33	2p	21	- 4.8 ± 8.53	2p
43	31.5 ± 11.9	2p	34	- 3.3 ± 6.07	2p
57	45.5 ± 21.9	2p	48	- 2.1 ± 5.4	2p
80	61.0 ± 22.8	2p	61	- 1.3 ± 5.9	2p
110	89.9 ± 32.4	2p	84	+ 0.1 ± 7.2	2p
			114	1.9 ± 5.9	2p
Diluted blood at room temperature			Diluted blood in ice bath		
11	21.8 ± 8.77	$2p < 0.001$	14	- 0.6 ± 1.8	$2p < 0.5$
24	27.9 ± 8.06	2p	28	0 ± 0	–
37	30.9 ± 10.03	2p	40	0 ± 0	–
51	31.0 ± 8.27	2p	54	0 ± 0	–
65	32.4 ± 9.7	2p	68	0 ± 0	–
87	33.7 ± 9.0	2p	90	0 ± 0	–
117	36.5 ± 9.2	2p	121	0 ± 0	–

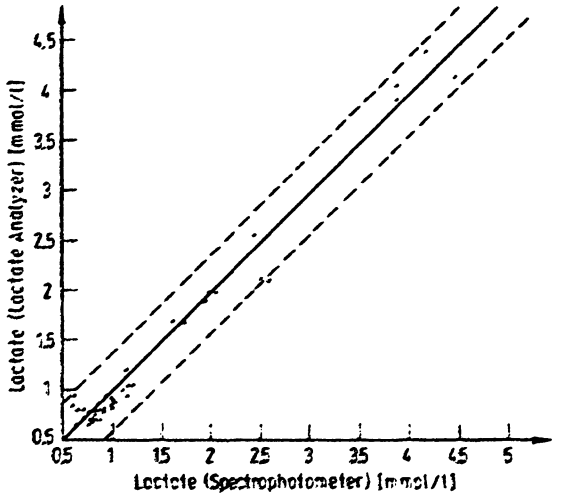

Fig. 5. Regression line for the correlation between the values of the Lactate Analyzer and the spectrophotometric method
—— regression line
---- 95 % confidence level data points

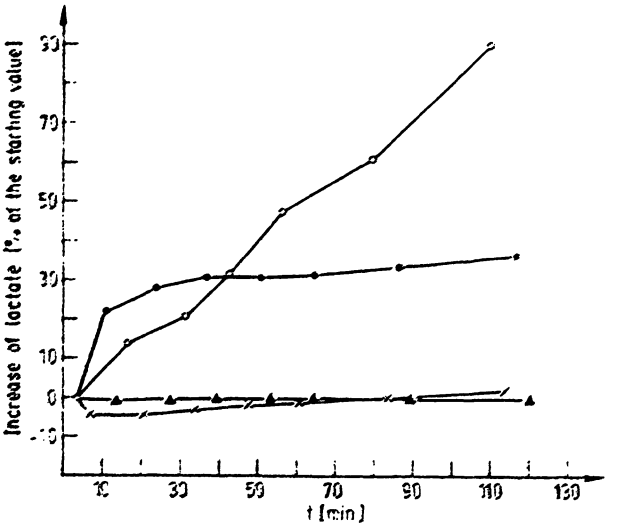

Fig. 6. Change of lactate concentration over 120 minutes in whole and diluted blood at room temperature and on ice
o——o whole blood at 25 °C
x——x whole blood on ice
•——• diluted blood at 25 °C
▲——▲ diluted blood on ice

was diluted with NaF containing buffer and stored in an ice bath, no change in lactate level was noted.

Table 4 shows the effect various reducing substances in the blood have on the measured lactate level. It can be seen that 11.9 mmol/l of uric acid increases the measured lactate level to 0.3 mmol/l. The other reducing substances showed smaller and clinically negligible effects. A slight diminution in apparent lactate levels occurs with some substances like cysteine, whereas when *L*-lactate is not present a small increase in current occurs which could be read as lactate. Table 5 shows that none of the drugs tested caused a change in the lactate reading. β-hydroxy-n-butyrate at concentrations of 0.1, 1.0 and 10 mmol/l showed no effect.

Tab. 4. Effect of various blood reducing substances on the reading of the lactate sensor

Substance	Normal blood level [mmol/l]	Concentration used [mmol/l]	Apparent change [mmol/l]
Ascorbic acid	0.04–0.14	0.14	0
β-Alanine	0.45	0.56	0
Creatinine	0.04	0.44	- 0.15
Cystine	0.08	0,08	- 0.1
Glycine	0.27	0.67	- 0.05
GSH	1.14	1.30	0
Urea	3.33	9.99	0
Uric acid	0.18–0.36	1.19	+ 0.3
Salicylate	?	2.17	0
Adrenaline	0.0014	0.014	0

Tab. 5. Influence of commonly used drugs in intensive care stations

Active substance	Concentration [mg/l]	Change in sensor reading [mmol/l]
Lanatosid C	0.13	0
Eraldin	2	0
p-Amino N 2 diethyl-aminoethylbenzamide HCl	170	0
Propanolol	4	0
Lidocaine	200	0
Diphenylhydantoin Na	10	0

Discussion

The blood handling tests indicate that at room temperature there is a continuous increase in the lactate concentration of whole blood over the observation time of 120 minutes. The lactate increase is due to continued lactate production by blood cells. When samples are placed in an ice bath, the metabolic processes are blocked and lactate production stops.

J. O. Westgard et al. (11) have shown that the lactate concentration is stable when whole blood is mixed with a sodium fluoride solution (2 g NaF/l) and centrifuged within 15 minutes after blood collection. They reported that whole blood containing NaF and stored at room temperature increased its lactate to 0.068 mmol/l in the first 15 minutes after sampling. Following 14 days of storage at 4 °C, there was a minimal increase of 0.026 mmol/l in the centrifuged samples. Their data indicated that the increase of lactate was due to continued lactate production by the blood cells in the first 15 minutes after blood collection.

In our tests, however, this effect is much less pronounced in diluted, not centrifuged, blood stored at room temperature. After a steep initial increase the curve for this series (fig. 6) levels off and shows no further marked lactate production during the next 90 minutes. The increase up to the plateau is, on the average, 30% above the initial value in the concentration range 0.6–2.0 mmol/l. The stabilizing effect of the NaF is visible in phase 2 of the curve but it does not prevent the fast initial increase. The utilization of NaF alone as a stabilizing agent is not sufficient in this particular situation.

Lactate concentration does not change if heparinized whole blood is diluted immediately after sampling with 0.1 mol/l phosphate buffer pH = 7.3 + 2 g/l NaF at a ratio of 1:5 (or 1:10) and the diluted blood is immediately placed in an ice bath. There were no deviations from the initial value in all 7 samples treated in this manner over the entire observation time of 2 hours. This interval should be sufficient for the daily routine application where it is not always possible to determine lactate immediately after sample collection.

The following practical possibilities exist for the optimum determination of lactate:

1. Heparinized whole blood should be diluted with buffer immediately after sampling and the lactate determined with the Lactate Analyzer without delay (< 3 minutes).
2. If it is not possible to determine the lactate immediately after sampling, one can either:

a) extend the time between blood withdrawal and measurement to at least 90 minutes by placing immediately the heparinized sample in an ice bath. In this manner no significant changes will be observed.

b) Dilute the sample with the appropriate buffer and place it also immediately in an ice bath. No change in lactate concentration will be observed at least for the next 120 minutes.

With the exception of unusually high levels of uric acid, none of the reducing substances found in blood interfere with the enzyme reaction used. Even the uric acid effect is minimal at commonly encountered uric acid levels. The drugs tested did not cause an effect. Drugs which potentiate or inhibit enzyme acitivity are a potential source of erroneous results. However, none has been found by us among drugs commonly used in situations where lactate determinations would be of clinical interest. Drug therapy might also affect the usable lifetime of the sensor without affecting the lactate results. This would be particularly true of drugs containing heavy metals such as mercury.

The Lactate Analyzer permits lactate determinations within 2–3 minutes of the time when blood is drawn. This is a decisive advantage for critically ill patients. The luxury of a rapid result is not possible with the spectrophotometric method since the classical method requires 1½ to 2 hours. However, certain automatic discrete sample analyzers now becoming available in large clinical laboratories require about the same amount of time per analysis as does this single purpose analyzer. A large discrete sample analyzer would not be as satisfactory as the lactate analyzer since it could not be placed in the patient area, thus producing a time delay between sampling and analysis which could cause an increase in lactate level.

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